PATENT
Atty. Docket No. 3218.2A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Xing SU et al.	Examiner
Serial No:	Group Art Unit:
Filing Date: Herewith	PRELIMINARY AMENDMENT
Title: COMPLEXITY MANAGEMENT AND ANALYSIS OF GENOMIC DNA	

Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-referenced application, please enter the following amendments and remarks.

In the Specification:

On page 1 of the specification, please delete the title and insert therefor:

Complexity Management and Analysis of Genomic DNA

At page 6, please delete the paragraph beginning on line 10 and insert the following paragraph:

An array comprises a solid support with nucleic acid probes attached to said support. Arrays typically comprise a plurality of different oligonucleotide probes that are coupled to a surface of a substrate in different known locations. These arrays, also described as "microarrays" or colloquially "chips" have been generally described in the art, for example, U.S. Pat. Nos. 5,143,854, 5445934, 5,744,305, 5,677,195 and PCT Patent Publication Nos. WO 90/15070 and 92/10092, each of which is incorporated by reference in its entirety for all purposes. These arrays may generally be produced using mechanical synthesis methods or light directed synthesis methods which incorporate a combination of photolithographic

methods and solid phase synthesis methods. See Fodor et al., Science, 251:767-777 (1991), Pirrung et al., U.S. Pat. No. 5,143,854 (see also PCT Application No. WO 90/15070) and Fodor et al., PCT Publication No. WO 92/10092 and U.S. Pat. No. 5,424,186, each of which is hereby incorporated in its entirety by reference for all purposes. Techniques for the synthesis of these arrays using mechanical synthesis methods are described in, e.g., U.S. Pat. No. 5,384,261, incorporated herein by reference in its entirety for all purposes. Although a planar array surface is preferred, the array may be fabricated on a surface of virtually any shape or even a multiplicity of surfaces. Arrays may be nucleic acids on beads, fibers such as fiber optics, glass or any other appropriate substrate, see US Patent Nos. 5,770,358, 5,789,162, 5,708,153 and 5,800,992 which are hereby incorporated in their entirety for all purposes. Arrays may be packaged in such a manner as to allow for diagnostics or other manipulation of in an all inclusive device, see for example, US Patent Nos. 5,856,174 and 5,922,591 incorporated in their entirety by reference for all purposes.

At page 10-11, please delete the paragraph beginning on page 10, line 20 and insert the following paragraph:

After isolation, adaptor sequences are ligated to the fragments. (Figure 2, Step 4) Adaptor sequences are generally oligonucleotides of at least 5 or 10 bases and preferably no more than 50 or 60 bases in length, however, adaptor sequences may be even longer, up to 100 or 200 bases depending upon the desired result. For example, if the desired outcome is to prevent amplification of a particular fragment, longer adaptor sequences designed to form stem loops or other tertiary structures may be ligated to the fragment. Adaptor sequences may be synthesized using any methods known to those of skill in the art. For the purposes of this invention they may, as options, comprise templates for PCR primers and/or tag or recognition sequences. The design and use of tag sequences is described in US Patent No. 5,800,992 and US Provisional Patent Application No. 60/140,359, filed 6/23/99, both of which are incorporated by reference for all purposes. Adaptor sequences may be ligated to either blunt end or sticky end DNA. Methods of ligation will be known to those of skill in the art and are described, for example, in Sambrook et al. Methods include DNase digestion to "nick" the DNA, ligation with ddNTP and the use of polymerase I to fill in gaps or any other methods described in the art.

At page 11, please delete the paragraph beginning on line 6 and insert the following paragraph:

Further complexity reduction is achieved by adding a specific nucleotide on the 3' end of the PCR primer as illustrated in Figure 3. The specific nucleotide further reduces the complexity of the resulting DNA pool because only those fragments which have been isolated after restriction enzyme digestion and contain the complement of the specific nucleotide(s) incorporated in the PCR primer will be amplified. Figure 3A depicts the results of hybridization to an array after enzyme digestion, ligation to an adaptor and PCR amplification. Figs. 3B and 3C depict the results of hybridization to an array after enzyme digestion, ligation to an adaptor and PCR amplification where the PCR primers incorporated specific nucleotides in the 3' end of the primer. In Fig. 3B the 5' and 3' primers have different specific nucleotides incorporated. In Fig. 3A the 5' and 3' primers have the same nucleotides incorporated. The level of complexity in the isolated pool can be varied depending upon the identity and number of nucleotides incorporated into the PCR primers. A number of embodiments of the present invention involve amplification by PCR. Any of these embodiments may be further modified to reduce complexity using the above disclosed technique.

At page 14 and 15, please delete the paragraph beginning on page 14, line 26 and insert the following paragraph:

In another embodiment, the step of complexity management comprises performing an arbitrarily primed polymerase chain reaction (AP PCR) upon the sample. AP PCR is described in US Patent No. 5,487,985 which is hereby incorporated by reference in its entirety for all purposes. Figure 7 depicts a schematic illustration of this embodiment. Performing AP PCR with random primers which have specific nucleotides incorporated into the primers produces a reduced representation of genomic DNA in a reproducible manner. Figure 8 shows the level of complexity reduction of human genomic DNA resulting from AP PCR with various primers. Column 1 lists the primer name. Column 2 lists the primer sequence. Column 3 lists the annealing temperature. Column 4 lists the polymerase used.

Column 5 lists the number correlated to a specific gene on the Hum6.8K GeneChip(R) probe array (Affymetrix, Inc. Santa Clara, Ca). Column 6 lists the percentage of the human genes on the Hum6.8K GeneChip(R) probe array found by fragments whose complexity has been reduced by this method. Figure 9 shows the reproducibility of AP PCR. Independently prepared samples were subjected to AP PCR using the same primers. The gel bands show that the level of reproducibility between the samples is very high.

At page 25-26, please delete the paragraph beginning on page 25, line 25 and insert the following paragraph:

The labeled DNA was then hybridized to an array in a hybridization mixture containing 80 ug labeled DNA, 160 ug human COT-1 DNA (GIBCO), 3.5 M tetramethylamonium cloride, 10 mM MES (pH 6.5), 0.01% Triton-100, 20 ug herring sperm DNA, 100 ug bovine serum albumin and 200 pM control oligomer at 44 °C for 40 hours on a rotisserie at 40 rpm. The arrays were then washed with 0.1 M MaCl in 10 mM MES at 44 °C for 30 minutes on a rotisserie at 40 rpm. The hybridized arrays were then stained with a staining solution [10 mM MES (ph 6.5), 1 M NaCl, 10 ug/ml steptaviden R-phycoerythrin, 0.5 mg/ml acetylated BSA, 0.01% Triton-100] at 40 °C for 15 minutes. The arrays were then washed with 6x SSPET [0.9 M NaCl, 60 mM NaH2PO4 (pH 7.4), 6 mM EDTA, 0.005 % Triton-100] on a GeneChip® Fluidics Station (Affymetrix, Inc., Santa Clara, CA) 10 times at 22 °C. The arrays were then anti-streptavidin antibody stained at 40 °C for 30 minutes with antibody solution [10mM MES (pH 6.5), 1 M NaCl, 10 ug/ml streptavidin R-phycoerythrin, 0.5 mg/ml actylated BSA, 0.01% Triton-100]. The arrays were then restained with staining solution for 15 minutes followed by 6X SSPET washing as above. The arrays were then scanned with a confocal scanner at 560 nm. The hybridization patterns were then screened for SNP detection with a computer program as described in D.G. Wang et al Science 280, 1077-1082, 1998. The results of the hybridization can be seen in Figures 8A and 8B.

At page 30, please delete the paragraph beginning on line 24 and insert the following paragraph:

PCR was performed in a MJ Research Tetrad thermocycler using an initial 10 minute denaturation at 95 °C, 35 cycles of a 0.5 minute denaturation at 94 °C, annealing for 0.5 minute at 57 °C and extension at 72 °C. This was followed by a final 5 minute extension at 72 °C.

At page 31, please delete the paragraph beginning on line 7 and insert the following paragraph:

The sample was then denatured at 95 °C for 10 minutes and allowed to reanneal by slowly cooling to room temperature.

At page 32, please delete the paragraph beginning on line 8 and insert the following paragraph:

PCR was performed in a MJ Research Tetrad Thermocycler using an initial 10 minute denaturation at 95 °C, 45 cycles of a 0.5 minute denaturation at 94 °C, annealing for 0.5 minute at 52 °C and extension at 72 °C for 1 minute. This was followed by a final 5 minute extension at 72 °C. The fragments were then labeled and hybridized to an array.

At page 33 and 34, please delete the paragraph beginning on page 33, line 28 and insert the following paragraph:

Correlation is performed for a population of individuals who have been tested for the presence or absence of a phenotypic trait of interest and for polymorphic markers sets. To perform such analysis, the presence or absence of a set of polymorphisms (i.e. a polymorphic set) is determined for a set of the individuals, some of whom exhibit a particular trait, and some of which exhibit lack of the trait. The alleles of each polymorphism of the set are then reviewed to determine whether the presence or absence of a particular allele is associated with the trait of interest. Correlation can be performed by standard statistical methods such as a chi-squared test and statistically significant correlations between polymorphic form(s) and phenotypic characteristics are noted. For example, it might be found that the presence of allele A1 at polymorphism A correlates with heart disease. As a further example, it might be

found that the combined presence of allele A1 at polymorphism A and allele B1 at polymorphism B correlates with increased milk production of a farm animal. (See, Beitz et al., US 5,292,639).

At page 36, please delete the paragraph beginning on line 24 and insert the following paragraph:

Similarly, some medications may be highly effective for only a very small percentage of the population while proving only slightly effective or even ineffective to a large percentage of patients. Pharmacogenomics allows pharamaceutical companies to predict which patients would be the ideal candidates for a particular drug, thereby dramatically reducing failure rates and providing greater incentive to companies to continue to conduct research into those drugs.

SEQUENCE LISTING

Transmitted herewith is a copy of the "Sequence Listing" (6 sheets) in paper form for the above identified patent application as required by 37 C.F.R. 1.821(c) and a copy of the "sequence listing" in computer readable form as required by 37 C.F.R. 1.821(e). As required by 37 C.F.R. 1.821(f), Applicant's attorney hereby states that the content of the "Sequence Listing" in paper form and the computer readable form of the "Sequence Listing" are the same.

Please insert the attached "Sequence Listing" comprising SEQ ID NOS: 1-22 into the above reference application.

In the Claims:

Please cancel Claims 1- 37 without prejudice and add new claims 39-173 as follows:

39. (New) A method of analyzing a first nucleic acid sample comprising:
providing said first nucleic acid sample;

obtaining a second nucleic acid sample by fragmenting said first nucleic acid sample to produce fragments, ligating adaptor sequences to said fragments, amplifying at least some of said fragments, and isolating said amplified fragments;

providing a nucleic acid array;

hybridizing said second nucleic acid sample to said array; and

analyzing a hybridization pattern resulting from said hybridization.

40. (New) The method of claim 39 wherein said second nucleic acid sample comprises at least 0.5 % of said first nucleic acid sample.

41. (New) The method of claim 39 wherein said second nucleic acid sample comprises at least 3 % of said first nucleic acid sample.

42. (New) The method of claim 39 wherein said second nucleic acid sample comprises at least 12 % of said first nucleic acid sample.

43. (New) The method of claim 39 wherein said second nucleic acid sample comprises at least 50 % of said first nucleic acid sample.

44. (New) The method of claim 39 wherein said first nucleic acid sample is DNA.

45. (New) The method of claim 39 wherein said first nucleic acid sample is genomic DNA.

46. (New) The method of claim 39 wherein said first nucleic acid sample is cDNA derived from RNA or mRNA.

47. (New) The method of claim 39 wherein the entire method is performed in a single reaction vessel.

48. (New) The method of claim 39 wherein said step of fragmenting the first nucleic acid sample comprises digestion with at least one restriction enzyme.

49. (New) The method of claim 39 wherein said step of fragmenting the first nucleic acid sample comprises digestion with a type IIs endonuclease.

50. (New) The method of claim 39 wherein said adaptor sequences comprise PCR primer template sequences.

51. (New) The method of claim 39 wherein said adaptor sequences comprise tag sequences.

52. (New) The method of claim 39 wherein said method for analyzing a first nucleic acid sample comprises determining whether the first nucleic acid sample contains sequence variations.

53. (New) The method of claim 52 wherein said sequence variations are single nucleotide polymorphisms (SNPs).

54. (New) The method of claim 39 wherein the nucleic acid array is designed to query DNA fragments which have been produced by the identical procedures used to obtain said second nucleic acid sample.

55. (New) The method of claim 54 wherein the sequences contained in said second nucleic acid sample are predetermined.

56. (New) The method of claim 54 wherein said sequences contained in said second nucleic acid sample are first determined by a computer system.

57. (New) The method of claim 53 wherein said second nucleic acid sample is obtainable by:

binding oligonucleotide probes containing a desired SNP sequence to magnetic beads to form probe-bead complexes;

hybridizing said probe-bead complexes to said first nucleic acid sample;

exposing said first nucleic acid sample to a single strand DNA nuclease to remove single stranded DNA thereby obtaining only DNA duplexes;

ligating a double stranded adaptor sequence comprising a restriction enzyme site to said DNA duplexes;

digesting said DNA duplexes with a restriction enzyme to release the magnetic bead; and

isolating the duplexes.

58. (New) The method of claim 57 wherein said restriction enzyme is a Class IIs endonuclease.

59. (New) The method of claim 53 wherein said second nucleic acid sample is obtainable by:

exposing the first nucleic acid sample to a mismatch binding protein;

employing a 3' to 5' exonuclease to remove one strand of double stranded DNA; and

employing a nuclease to remove single stranded DNA.

60. (New) A method of screening for DNA sequence variations in an individual comprising:

providing a first nucleic acid sample from said individual;

obtaining a second nucleic acid sample by fragmenting said first nucleic acid sample to produce fragments, ligating adaptor sequences to said fragments, amplifying at least some of said fragments, and isolating said amplified fragments;

providing a nucleic acid array wherein said array comprises probes designed to interrogate for DNA sequence variations;

hybridizing said second nucleic acid sample to said array;

generating a hybridization pattern resulting from said hybridization; and

determining the presence or absence of DNA sequence variations in the individual based upon an analysis of the hybridization pattern.

61. (New) The method of claim 60 wherein said sequence variation is a single nucleotide polymorphism (SNP).

62. (New) The method of claim 61 wherein said SNP is associated with a disease.

63. (New) The method of claim 61 wherein said SNP is associated with the efficacy of a drug.

64. (New) A method of analyzing a first nucleic acid sample comprising:

providing said first nucleic acid sample;

obtaining a second nucleic acid sample by fragmenting said first nucleic acid sample to produce fragments, denaturing said fragments, allowing at least some of said fragments to reanneal to form double stranded DNA sequences and removing said double stranded DNA sequences thus isolating said single stranded fragments;

providing a nucleic acid array;

hybridizing said second nucleic acid sample to said array; and

analyzing a hybridization pattern resulting from said hybridization.

65. (New) The method of claim 64 further comprising the step of amplifying the isolated fragments.

66. (New) The method of claim 64 wherein said step of amplifying is performed by a polymerase chain reaction (PCR).

67. (New) The method of claim 64 wherein said second nucleic acid sample comprises at least 0.5 % of said first nucleic acid sample.

68. (New) The method of claim 64 wherein said second nucleic acid sample comprises at least 3 % of said first nucleic acid sample.

69. (New) The method of claim 64 wherein said second nucleic acid sample comprises at least 12 % of said first nucleic acid sample.

70. (New) The method of claim 64 wherein said second nucleic acid sample comprises at least 50 % of said first nucleic acid sample.

71. (New) The method of claim 64 wherein said first nucleic acid sample is DNA.

72. (New) The method of claim 64 wherein said first nucleic acid sample is genomic DNA.

73. (New) The method of claim 64 wherein said first nucleic acid sample is cDNA derived from RNA or mRNA.

74. (New) The method of claim 64 wherein the entire method is performed in a single reaction vessel.

75. (New) The method of claim 64 wherein said step of fragmenting the first nucleic acid sample comprises digestion with at least one restriction enzyme.

76. (New) The method of claim 64 wherein said step of fragmenting the first nucleic acid sample comprises digestion with a type IIs endonuclease.

77. (New) The method of claim 64 wherein said method for analyzing a first nucleic acid sample comprises determining whether the first nucleic acid sample contains sequence variations.

78. (New) The method of claim 77 wherein said sequence variations are single nucleotide polymorphisms (SNPs).

79. (New) The method of claim 64 wherein the nucleic acid array is designed to query DNA fragments which have been produced by the identical procedures used to obtain said second nucleic acid sample.

80. (New) The method of claim 79 wherein the sequences contained in said second nucleic acid sample are predetermined.

81. (New) The method of claim 79 wherein said sequences contained in said second nucleic acid sample are first determined by a computer system.

82. (New) The method of claim 78 wherein said second nucleic acid sample is obtainable by:

binding oligonucleotide probes containing a desired SNP sequence to magnetic beads to form probe-bead complexes;

hybridizing said probe-bead complexes to said first nucleic acid sample;

exposing said first nucleic acid sample to a single strand DNA nuclease to remove single stranded DNA thereby obtaining only DNA duplexes;

ligating a double stranded adaptor sequence comprising a restriction enzyme site to said DNA duplexes;

digesting said DNA duplexes with a restriction enzyme to release the magnetic bead; and

isolating the duplexes.

83. (New) The method of claim 82 wherein said restriction enzyme is a Class IIs endonuclease.

84. (New) The method of claim 78 wherein said second nucleic acid sample is obtainable by:

exposing the first nucleic acid sample to a mismatch binding protein;

employing a 3' to 5' exonuclease to remove one strand of double stranded DNA; and

employing a nuclease to remove single stranded DNA.

85. (New) A method of screening for DNA sequence variations in an individual comprising:

providing a first nucleic acid sample from said individual;

obtaining a second nucleic acid sample by fragmenting said first nucleic acid sample to produce fragments, denaturing said fragments, allowing some of said

fragments to reanneal to form double stranded DNA sequences and removing said double stranded DNA sequences thus isolating said single stranded fragments;

providing a nucleic acid array wherein said array comprises probes designed to interrogate for DNA sequence variations;

hybridizing said second nucleic acid sample to said array;

generating a hybridization pattern resulting from said hybridization; and

determining the presence or absence of DNA sequence variations in the individual based upon an analysis of the hybridization pattern.

86. (New) The method of claim 85 wherein said sequence variation is a single nucleotide polymorphism (SNP).

87. (New) The method of claim 86 wherein said SNP is associated with a disease.

88. (New) The method of claim 86 wherein said SNP is associated with the efficacy of a drug.

89. (New) A method of analyzing a first nucleic acid sample comprising:

providing said first nucleic acid sample;

obtaining a second nucleic acid sample by amplifying said first nucleic acid sample by arbitrarily primed PCR to produce an amplification product and isolating said amplification product;

providing a nucleic acid array;

hybridizing said second nucleic acid sample to said array; and

analyzing a hybridization pattern resulting from said hybridization.

90. (New) The method of claim 89 wherein said second nucleic acid sample comprises at least 0.5 % of said first nucleic acid sample.

91. (New) The method of claim 89 wherein said second nucleic acid sample comprises at least 3 % of said first nucleic acid sample.

92. (New) The method of claim 89 wherein said second nucleic acid sample comprises at least 12 % of said first nucleic acid sample.

93. (New) The method of claim 89 wherein said second nucleic acid sample comprises at least 50 % of said first nucleic acid sample.

94. (New) The method of claim 89 wherein said first nucleic acid sample is DNA.

95. (New) The method of claim 89 wherein said first nucleic acid sample is genomic DNA.

96. (New) The method of claim 89 wherein said first nucleic acid sample is cDNA derived from RNA or mRNA.

97. (New) The method of claim 89 wherein the entire method is performed in a single reaction vessel.

98. (New) The method of claim 89 wherein said step of fragmenting the first nucleic acid sample comprises digestion with at least one restriction enzyme.

99. (New) The method of claim 89 wherein said step of fragmenting the first nucleic acid sample comprises digestion with a type IIs endonuclease.

100. (New) The method of claim 89 wherein said method for analyzing a first nucleic acid sample comprises determining whether the first nucleic acid sample contains sequence variations.

101. (New) The method of claim 100 wherein said sequence variations are single nucleotide polymorphisms (SNPs).

102. (New) The method of claim 89 wherein the nucleic acid array is designed to query DNA fragments which have been produced by the identical procedures used to obtain said second nucleic acid sample.

103. (New) The method of claim 102 wherein the sequences contained in said second nucleic acid sample are predetermined.

104. (New) The method of claim 102 wherein said sequences contained in said second nucleic acid sample are first determined by a computer system.

105. (New) The method of claim 101 wherein said second nucleic acid sample is obtainable by:

binding oligonucleotide probes containing a desired SNP sequence to magnetic beads to form probe-bead complexes;

hybridizing said probe-bead complexes to said first nucleic acid sample;

exposing said first nucleic acid sample to a single strand DNA nuclease to remove single stranded DNA thereby obtaining only DNA duplexes;

ligating a double stranded adaptor sequence comprising a restriction enzyme site to said DNA duplexes;

digesting said DNA duplexes with a restriction enzyme to release the magnetic bead; and

isolating the duplexes.

106. (New) The method of claim 105 wherein said restriction enzyme is a Class IIs endonuclease.

107. (New) The method of claim 101 wherein said second nucleic acid sample is obtainable by:

exposing the first nucleic acid sample to a mismatch binding protein;

employing a 3' to 5' exonuclease to remove one strand of double stranded DNA; and

employing a nuclease to remove single stranded DNA.

108. (New) A method of screening for DNA sequence variations in an individual comprising:

providing a first nucleic acid sample from said individual;

obtaining a second nucleic acid sample by amplifying said first nucleic acid sample by arbitrarily primed PCR to produce an amplification product and isolating said amplification product ;

providing a nucleic acid array wherein said array comprises probes designed to interrogate for DNA sequence variations;

hybridizing said second nucleic acid sample to said array;

generating a hybridization pattern resulting from said hybridization; and

determining the presence or absence of DNA sequence variations in the individual based upon an analysis of the hybridization pattern.

109. (New) The method of claim 108 wherein said sequence variation is a single nucleotide polymorphism (SNP).

110. (New)The method of claim 109 wherein said SNP is associated with a disease.

111. (New) The method of claim 109 wherein said SNP is associated with the efficacy of a drug.

112. (New) A method of analyzing a first nucleic acid sample comprising:

providing said first nucleic acid sample;

obtaining a second nucleic acid sample by fragmenting said first nucleic acid sample to produce fragments, hybridizing said fragments to an oligonucleotide probe bound to a solid support, and isolating said hybridized fragments;

providing a nucleic acid array;

hybridizing said second nucleic acid sample to said array; and

analyzing a hybridization pattern resulting from said hybridization.

113. (New) The method of claim 112 further comprising the step of amplifying the isolated fragments.

114. (New) The method of claim 112 wherein said step of amplifying is performed by a polymerase chain reaction (PCR).

115. (New) The method of claim 112 wherein said second nucleic acid sample comprises at least 0.5 % of said first nucleic acid sample.

116. (New) The method of claim 112 wherein said second nucleic acid sample comprises at least 3 % of said first nucleic acid sample.

117. (New) The method of claim 112 wherein said second nucleic acid sample comprises at least 12 % of said first nucleic acid sample.

118. (New) The method of claim 112 wherein said second nucleic acid sample comprises at least 50 % of said first nucleic acid sample.

119. (New) The method of claim 112 wherein said first nucleic acid sample is DNA.

120. (New) The method of claim 112 wherein said first nucleic acid sample is genomic DNA.

121. (New) The method of claim 112 wherein said first nucleic acid sample is cDNA derived from RNA or mRNA.

122. (New) The method of claim 112 wherein the entire method is performed in a single reaction vessel.

123. (New) The method of claim 112 wherein said step of fragmenting the first nucleic acid sample comprises digestion with at least one restriction enzyme.

124. (New) The method of claim 112 wherein said step of fragmenting the first nucleic acid sample comprises digestion with a type IIs endonuclease.

125. (New) The method of claim 112 wherein said solid support is a magnetic bead.

126. (New) The method of claim 112 wherein said method for analyzing a first nucleic acid sample comprises determining whether the first nucleic acid sample contains sequence variations.

127. (New) The method of claim 126 wherein said sequence variations are single nucleotide polymorphisms (SNPs).

128. (New) The method of claim 112 wherein the nucleic acid array is designed to query DNA fragments which have been produced by the identical procedures used to obtain said second nucleic acid sample.

129. (New) The method of claim 128 wherein the sequences contained in said second nucleic acid sample are predetermined.

130. (New) The method of claim 128 wherein said sequences contained in said second nucleic acid sample are first determined by a computer system.

131. (New) The method of claim 127 wherein said second nucleic acid sample is obtainable by:

binding oligonucleotide probes containing a desired SNP sequence to magnetic beads to form probe-bead complexes;

hybridizing said probe-bead complexes to said first nucleic acid sample;

exposing said first nucleic acid sample to a single strand DNA nuclease to remove single stranded DNA thereby obtaining only DNA duplexes;

ligating a double stranded adaptor sequence comprising a restriction enzyme site to said DNA duplexes;

digesting said DNA duplexes with a restriction enzyme to release the magnetic bead; and

isolating the duplexes.

132. (New) The method of claim 131 wherein said restriction enzyme is a Class IIs endonuclease.

133. (New) The method of claim 127 wherein said second nucleic acid sample is obtainable by:

exposing the first nucleic acid sample to a mismatch binding protein;

employing a 3' to 5' exonuclease to remove one strand of double stranded DNA; and

employing a nuclease to remove single stranded DNA.

134. (New) A method of screening for DNA sequence variations in an individual comprising:

providing a first nucleic acid sample from said individual;

obtaining a second nucleic acid sample by fragmenting said first nucleic acid sample to produce fragments, hybridizing said fragments to an oligonucleotide probe bound to a solid support, and isolating said hybridized fragments;

providing a nucleic acid array wherein said array comprises probes designed to interrogate for DNA sequence variations;

hybridizing said second nucleic acid sample to said array;

generating a hybridization pattern resulting from said hybridization; and

determining the presence or absence of DNA sequence variations in the individual based upon an analysis of the hybridization pattern.

135. (New) The method of claim 134 wherein said sequence variation is a single nucleotide polymorphism (SNP).

136. (New) The method of claim 135 wherein said SNP is associated with a disease.

137. (New) The method of claim 135 wherein said SNP is associated with the efficacy of a drug.

138. (New) A method of analyzing a first nucleic acid sample comprising:

providing said first nucleic acid sample;

obtaining a second nucleic acid sample by fragmenting said first nucleic acid sample to produce fragments, binding said fragments to a mismatch binding protein, and isolating said bound fragments;

providing a nucleic acid array;

hybridizing said second nucleic acid sample to said array; and

analyzing a hybridization pattern resulting from said hybridization..

139. (New) The method of claim 138 further comprising the step of amplifying the isolated fragments.

140. (New) The method of claim 138 wherein said step of amplifying is performed by a polymerase chain reaction (PCR).

141. (New) The method of claim 138 wherein said second nucleic acid sample comprises at least 0.5 % of said first nucleic acid sample.

142. (New) The method of claim 138 wherein said second nucleic acid sample comprises at least 3 % of said first nucleic acid sample.

143. (New) The method of claim 138 wherein said second nucleic acid sample comprises at least 12 % of said first nucleic acid sample.

144. (New) The method of claim 138 wherein said second nucleic acid sample comprises at least 50 % of said first nucleic acid sample.

145. (New) The method of claim 138 wherein said first nucleic acid sample is DNA.

146. (New) The method of claim 138 wherein said first nucleic acid sample is genomic DNA.

147. (New) The method of claim 138 wherein said first nucleic acid sample is cDNA derived from RNA or mRNA.

148. (New) The method of claim 138 wherein the entire method is performed in a single reaction vessel.

149. (New) The method of claim 138 wherein said step of fragmenting the first nucleic acid sample comprises digestion with at least one restriction enzyme.

150. (New) The method of claim 138 wherein said step of fragmenting the first nucleic acid sample comprises digestion with a type IIs endonuclease.

151. (New) The method of claim 138 wherein said mismatch binding protein is bound to a magnetic bead.

152. (New) The method of claim 138 wherein said method for analyzing a first nucleic acid sample comprises determining whether the first nucleic acid sample contains sequence variations.

153. (New) The method of claim 152 wherein said sequence variations are single nucleotide polymorphisms (SNPs).

154. (New) The method of claim 138 wherein the nucleic acid array is designed to query DNA fragments which have been produced by the identical procedures used to obtain said second nucleic acid sample.

155. (New) The method of claim 154 wherein the sequences contained in said second nucleic acid sample are predetermined.

156. (New) The method of claim 154 wherein said sequences contained in said second nucleic acid sample are first determined by a computer system.

157. (New) The method of claim 153 wherein said second nucleic acid sample is obtainable by:

binding oligonucleotide probes containing a desired SNP sequence to magnetic beads to form probe-bead complexes;

hybridizing said probe-bead complexes to said first nucleic acid sample;

exposing said first nucleic acid sample to a single strand DNA nuclease to remove single stranded DNA thereby obtaining only DNA duplexes;

ligating a double stranded adaptor sequence comprising a restriction enzyme site to said DNA duplexes;

digesting said DNA duplexes with a restriction enzyme to release the magnetic bead; and

isolating the duplexes.

158. (New) The method of claim 157 wherein said restriction enzyme is a Class IIs endonuclease.

159. (New) The method of claim 153 wherein said second nucleic acid sample is obtainable by:

exposing the first nucleic acid sample to a mismatch binding protein;

employing a 3' to 5' exonuclease to remove one strand of double stranded DNA; and

employing a nuclease to remove single stranded DNA.

160. (New) A method of screening for DNA sequence variations in an individual comprising:

providing a first nucleic acid sample from said individual;

obtaining a second nucleic acid sample by fragmenting said first nucleic acid sample to produce fragments, binding said fragments to a mismatch binding protein, and isolating said bound fragments;

providing a nucleic acid array wherein said array comprises probes designed to interrogate for DNA sequence variations;

hybridizing said second nucleic acid sample to said array;

generating a hybridization pattern resulting from said hybridization; and

determining the presence or absence of DNA sequence variations in the individual based upon an analysis of the hybridization pattern.

161. (New) The method of claim 160 wherein said sequence variation is a single nucleotide polymorphism (SNP).

162. (New) The method of claim 161 wherein said SNP is associated with a disease.

163. (New) The method of claim 161 wherein said SNP is associated with the efficacy of a drug.

164. (New) A method of screening for DNA sequence variations in a population of individuals comprising:

providing a first nucleic acid sample from each of said individuals;

providing a second nucleic acid sample by fragmenting said first nucleic acid sample to produce fragments, ligating adaptor sequences to said fragments, amplifying at least some of said fragments, and isolating said amplified fragments;

providing a plurality of identical nucleic acid arrays wherein said arrays comprise probes which are designed to interrogate for DNA sequence variations;

hybridizing each of said second nucleic acid samples to one of said plurality of identical arrays; and

generating a plurality of hybridization patterns resulting from said hybridizations; and

analyzing the hybridization patterns to determine the presence or absence of sequence variation in the population of individuals.

165. (New) The method of claim 164 wherein said sequence variation is a single nucleotide polymorphism.

166. (New) A method of screening for DNA sequence variations in a population of individuals comprising:

providing a first nucleic acid sample from each of said individuals;

providing a second nucleic acid sample by fragmenting said first nucleic acid sample to produce fragments, denaturing said fragments, allowing some of said fragments to reanneal to form double stranded DNA sequences and removing said double stranded DNA sequences thus isolating said single stranded fragments;

providing a plurality of identical nucleic acid arrays wherein said arrays comprise probes which are designed to interrogate for DNA sequence variations;

hybridizing each of said second nucleic acid samples to one of said plurality of identical arrays; and

generating a plurality of hybridization patterns resulting from said hybridizations; and

analyzing the hybridization patterns to determine the presence or absence of sequence variation in the population of individuals.

167. (New) The method of claim 166 wherein said sequence variation is a single nucleotide polymorphism.

168. (New) A method of screening for DNA sequence variations in a population of individuals comprising:

providing a first nucleic acid sample from each of said individuals;

providing a second nucleic acid sample by amplifying said first nucleic acid sample by arbitrarily primed PCR to produce an amplification product and isolating said amplification product ;

providing a plurality of identical nucleic acid arrays wherein said arrays comprise probes which are designed to interrogate for DNA sequence variations;

hybridizing each of said second nucleic acid samples to one of said plurality of identical arrays; and

generating a plurality of hybridization patterns resulting from said hybridizations; and

analyzing the hybridization patterns to determine the presence or absence of sequence variation in the population of individuals.

169. (New) The method of claim 168 wherein said sequence variation is a single nucleotide polymorphism.

170. (New) A method of screening for DNA sequence variations in a population of individuals comprising:

providing a first nucleic acid sample from each of said individuals;

providing a second nucleic acid sample by fragmenting said first nucleic acid sample to produce fragments, hybridizing said fragments to an oligonucleotide probe bound to a solid support, and isolating said hybridized fragments;

providing a plurality of identical nucleic acid arrays wherein said arrays comprise probes which are designed to interrogate for DNA sequence variations;

hybridizing each of said second nucleic acid samples to one of said plurality of identical arrays; and

generating a plurality of hybridization patterns resulting from said hybridizations; and

analyzing the hybridization patterns to determine the presence or absence of sequence variation in the population of individuals.

171. (New) The method of claim 170 wherein said sequence variation is a single nucleotide polymorphism.

172. (New) A method of screening for DNA sequence variations in a population of individuals comprising:

providing a first nucleic acid sample from each of said individuals;

providing a second nucleic acid sample by fragmenting said first nucleic acid sample to produce fragments, binding said fragments to a mismatch binding protein, and isolating said bound fragments;

providing a plurality of identical nucleic acid arrays wherein said arrays comprise probes which are designed to interrogate for DNA sequence variations;

hybridizing each of said second nucleic acid samples to one of said plurality of identical arrays; and

generating a plurality of hybridization patterns resulting from said hybridizations; and

analyzing the hybridization patterns to determine the presence or absence of sequence variation in the population of individuals.

173. (New) The method of claim 172 wherein said sequence variation is a single nucleotide polymorphism.

REMARKS

Please see Attachment A for marked up versions of the deleted paragraphs. Claims 1-37 have been canceled, and new claims 39-173 have been added. The canceled claims are included in Attachment B. Examination of the subject application, as amended, is respectfully requested.

Respectfully submitted,

12 July 2001

Wei Zhou
Reg. No. 44,419

Legal Department
Affymetrix, Inc.
3380 Central Expressway
Santa Clara, CA 95051
Tel: 408/731-5000
Fax: 408/731-5392

Attachment A: Marked up paragraphs according to 1.121(b)(iii):

Page 1, title:

Complexity [Management] Management and Analysis of Genomic DNA

Page 6, paragraph beginning on line 10:

An array comprises a solid support with nucleic acid probes attached to said support. Arrays typically comprise a plurality of different oligonucleotide probes that are coupled to a surface of a substrate in different known locations. These arrays, also described as "microarrays" or colloquially "chips" have been generally described in the art, for example, U.S. Pat. Nos. 5,143,854, 5445934, 5,744,305, 5,677,195 and PCT Patent Publication Nos. WO 90/15070 and 92/10092[. Each], each of which is incorporated by reference in its entirety for all purposes. These arrays may generally be produced using mechanical synthesis methods or light directed synthesis methods which incorporate a combination of photolithographic methods and solid phase synthesis methods. See Fodor et al., Science, 251:767-777 (1991), Pirrung et al., U.S. Pat. No. 5,143,854 (see also PCT Application No. WO 90/15070) and Fodor et al., PCT Publication No. WO 92/10092 and U.S. Pat. No. 5,424,186, each of which is hereby incorporated in its entirety by reference for all purposes. Techniques for the synthesis of these arrays using mechanical synthesis methods are described in, e.g., U.S. Pat. No. 5,384,261, incorporated herein by reference in its entirety for all purposes. Although a planar array surface is preferred, the array may be fabricated on a surface of virtually any shape or even a multiplicity of surfaces. Arrays may be nucleic acids on beads, fibers such as fiber optics, glass or any other appropriate substrate, see US Patent Nos. 5,770,358, 5,789,162, 5,708,153 and 5,800,992 which are hereby incorporated in their entirety for all purposes. Arrays may be packaged in such a manner as to allow for diagnostics or other manipulation of in an all inclusive device, see for example, US Patent Nos. 5,856,174 and 5,922,591 incorporated in their entirety by reference for all purposes.

Page 10-11, paragraph beginning on page 10, line 20:

After isolation, adaptor sequences are ligated to the fragments. (Figure 2, Step 4) Adaptor sequences are generally oligonucleotides of at least 5 or 10 bases and preferably no more than 50 or 60 bases in length, however, adaptor sequences may be even longer, up to 100 or 200 bases depending upon the desired result. For example, if the desired outcome is to prevent amplification of a particular fragment, longer adaptor sequences designed to form stem loops or other tertiary structures may be ligated to the fragment. Adaptor sequences may be synthesized using any methods known to those of skill in the art. For the purposes of this invention they may, as options, comprise templates for PCR primers and/or tag or recognition sequences. The design and use of tag sequences is described in US Patent No. 5,800,992 and US Provisional Patent Application No. 60/140,359, filed 6/23/99[. Both] ,both of which are incorporated by reference for all purposes. Adaptor sequences may be ligated to either blunt end or sticky end DNA. Methods of ligation will be known to those of skill in the art and are described, for example, in Sambrook et al. Methods include DNase digestion to "nick"

the DNA, ligation with ddNTP and the use of polymerase I to fill in gaps or any other methods described in the art.

Page 11, paragraph beginning on line 6:

Further complexity reduction is achieved by adding a specific nucleotide on the 3' [5'] end of the PCR primer as illustrated in Figure 3. The specific nucleotide further reduces the complexity of the resulting DNA pool because only those fragments which have been isolated after restriction enzyme digestion and contain the complement of the specific nucleotide(s) incorporated in the PCR primer will be amplified. Figure 3A depicts the results of hybridization to an array after enzyme digestion, ligation to an adaptor and PCR amplification. Figs. 3B and 3C depict the results of hybridization to an array after enzyme digestion, ligation to an adaptor and PCR amplification where the PCR primers incorporated specific nucleotides in the 3' [5'] end of the primer. In Fig. 3B the 5' and 3' primers have different specific nucleotides incorporated. In Fig. 3A the 5' and 3' primers have the same nucleotides incorporated. The level of complexity in the isolated pool can be varied depending upon the identity and number of nucleotides incorporated into the PCR primers. A number of embodiments of the present invention involve amplification by PCR. Any of these embodiments may be further modified to reduce complexity using the above disclosed technique.

Page 14-15, paragraph beginning on page 14, line 26

In another embodiment, the step of complexity management comprises performing an arbitrarily primed polymerase chain reaction (AP PCR) upon the sample. AP PCR is described in US Patent No. 5,487,985 which is hereby incorporated by reference in its entirety for all purposes. Figure 7 depicts a schematic illustration of this embodiment. Performing AP PCR with random primers which have specific nucleotides incorporated into the primers produces a reduced representation of genomic DNA in a reproducible manner. Figure 8 shows the level of complexity reduction of human genomic DNA resulting from AP PCR with various primers. Column 1 lists the primer name. Column 2 lists the primer sequence. Column 3 lists the annealing temperature. Column 4 lists the polymerase used. Column 5 lists the number correlated to a specific gene on the Hum6.8K GeneChip(R) probe array (Affymetrix, Inc. Santa Clara, Ca). Column 6 lists the percentage of the human genes on the Hum6.8K GeneChip(R) probe array found by fragments whose complexity has been reduced by this method. Figure 9 shows the reproducibility of AP PCR. Independently prepared samples [preps] were subjected to AP PCR using the same primers. The gel bands show that the level of reproducibility between the samples is very high.

Page 25-26, paragraph beginning on page 25, line 25:

The labeled DNA was then hybridized to an array in a hybridization mixture containing 80 ug labeled DNA, 160 ug human COT-1 DNA (GIBCO), 3.5 M tetramethylamonium cloride, 10 mM MES (pH 6.5), 0.01% Triton-100, 20 ug herring sperm DNA, 100 ug bovine serum albumin and 200 pM control oligomer at 44 °C for 40 hours on a rotisserie at 40 rpm. The arrays were then washed with 0.1 M MaCl in 10 mM MES at 44 °C for 30 minutes on a rotisserie at 40 rpm. The hybridized arrays were then stained with a staining solution [10 mM MES (ph 6.5), 1 M NaCl, 10 ug/ml steptaviden R-phycoerythrin, 0.5 mg/ml acetylated BSA, 0.01% Triton-100] at 40 °C for 15 minutes. The arrays were then washed with 6x SSPET [0.9 M NaCl, 60 mM NaH2PO4 (pH 7.4), 6 mM EDTA, 0.005 % Triton-100] on a GeneChip® Fluidics Station (Affymetrix, Inc., Santa Clara, CA) 10 times at 22 °C. The arrays were then anti-streptavidin antibody stained at 40 °C for 30 minutes with antibody solution [10mM MES (pH 6.5), 1 M NaCl, 10 ug/ml streptavidin R-phycoerythrin, 0.5 mg/ml actylated BSA, 0.01% Triton-100]. The arrays [are] were then restained with staining solution for 15 minutes followed by 6X SSPET washing as above. The arrays [are] were then scanned with a confocal scanner at 560 nm. The hybridization patterns were then screened for SNP detection with a computer program as described in D.G. Wang et al Science 280, 1077-1082, 1998. The results of the hybridization can be seen in Figures 8A and 8B.

Page 30, paragraph beginning on line 24:

PCR was performed in a MJ Research Tetrad thermocycler using an initial 10 minute denaturation at 95 °C, 35 cycles of a 0.5 minute denaturation at 94 °C, annealing for 0.5 minute at 57 °C and extension at 72 °C. This [is] was followed by a final 5 minute extension at 72 °C.

Page 31, paragraph beginning on line 7:

The sample was then denatured at 95 °C for 10 minutes and allowed to reanneal by slowly cooling to room temperature.

Page 32, paragraph beginning on line 8:

PCR was performed in a MJ Research Tetrad Thermocycler using an initial 10 minute denaturation at 95 °C, 45 cycles of a 0.5 minute denaturation at 94 °C, annealing for 0.5 minute at 52 °C and extension at 72 °C for 1 minute. This [is] was followed by a final 5 minute extension at 72 °C. The fragments were then labeled and hybridized to an array.

Page 33-34, paragraph beginning on page 33, line 28:

Correlation is performed for a population of individuals who have been tested for the presence or absence of a phenotypic trait of interest and for polymorphic markers sets. To perform such analysis, the presence or absence of a set of polymorphisms (i.e. a polymorphic set) is determined for a set of the individuals, some of whom exhibit a particular trait, and some of which exhibit lack of the trait. The alleles of each

polymorphism of the set are then reviewed to determine whether the presence or absence of a particular allele is associated with the trait of interest. Correlation can be performed by standard statistical methods such as a [6-squared] chi-squared test and statistically significant correlations between polymorphic form(s) and phenotypic characteristics are noted. For example, it might be found that the presence of allele A1 at polymorphism A correlates with heart disease. As a further example, it might be found that the combined presence of allele A1 at polymorphism A and allele B1 at polymorphism B correlates with increased milk production of a farm animal. (See, Beitz et al., US 5,292,639).

Page 36, paragraph beginning on line 24:

Similarly, some medications may be highly effective for only a very small percentage of the population while proving only slightly effective or even ineffective to a large percentage of patients. Pharmacogenomics allows pharamaceutical companies to predict which patients would be the ideal [candidate] candidates for a particular drug, thereby dramatically reducing failure rates and providing greater incentive to companies to continue to conduct research into those drugs.

Attachment B: Canceled Claims:

1. (Canceled) A method of analyzing a first nucleic sample comprising:

providing said first nucleic acid sample;

reproducibly reducing the complexity of said first nucleic acid sample to produce a second nucleic acid sample which may comprise a plurality of non-identical sequences whereby said second nucleic acid sample is obtainable by:

fragmenting said first nucleic acid sample to produce fragments and ligating adaptor sequences to said fragments;

fragmenting said first nucleic acid sample to produce fragments, denaturing said fragments, allowing some of said fragments to reanneal to form double stranded DNA sequences and removing said double stranded DNA sequences.

amplification by arbitrarily primed PCR;

hybridizing said first nucleic acid sample to an oligonucleotide probe bound to a solid support;

hybridizing said first nucleic acid sequence to a mismatch binding protein;

providing a nucleic acid array;

hybridizing said second nucleic acid sample to said array; and

analyzing a hybridization pattern resulting from said hybridization.

2. (Canceled) The method of claim 1 wherein said second nucleic acid sample comprises at least 0.5 % of said nucleic acid sample

3. (Canceled) The method of claim 1 wherein said second nucleic acid sample comprises at least 3 % of said nucleic acid sample

4. (Canceled) The method of claim 1 wherein said second nucleic acid sample comprises at least 12 % of said nucleic acid sample
at least 12%

5. (Canceled) The method of claim 1 wherein said second nucleic acid sample comprises at least 50 % of said nucleic acid sample

6. (Canceled) The method of claim 1 wherein each of said non-identical sequences differs from the other non-identical sequences by at least 5 nucleic acid bases.

7. (Canceled) The method of claim 1 wherein each of said non-identical sequences differs from the other non-identical sequences by at least 10 nucleic acid bases.

8. (Canceled) The method of claim 1 wherein each of said non-identical sequences differs from the other non-identical sequences by at least 50 nucleic acid bases.

9. (Canceled) The method of claim 1 wherein each of said non-identical sequences differs from the other non-identical sequences by at least 1000 nucleic acid bases.

10. (Canceled) The method of claim 1 wherein said NA sample is DNA.

11. (Canceled) The method of claim 1 wherein said NA sample is genomic DNA.

12. (Canceled) The method of claim 1 wherein said first nucleic acid sample is cDNA derived from RNA or mRNA.

13. (Canceled) The method of claim 1 further comprising the step of amplifying at least one of the non-identical sequences in said second nucleic acid sample.

14. (Canceled) The method of claim 13 wherein said step of amplifying is performed by a polymerase chain reaction (PCR).

15. (Canceled) The method of claim 1 wherein the entire method is performed in a single reaction vessel.

16. (Canceled) The method of claim 1 wherein said step of fragmenting the first nucleic acid sample comprises digestion with at least one restriction enzyme.

17. (Canceled) The method of claim 1 wherein said step of fragmenting the first nucleic acid sample comprises digestion with a type IIs endonuclease.

18. (Canceled) The method of claim 1 wherein said adaptor sequences comprise PCR primer template sequences.

19. (Canceled) The method of claim 1 wherein said adaptor sequences comprise tag sequences.

20. (Canceled) The method of claim 1 wherein said solid support is a magnetic bead.

21. (Canceled) The method of claim 1 wherein said mismatch binding protein is bound to a magnetic bead.

22. (Canceled) The method of claim 1 wherein said method for analyzing a nucleic acid sample comprises determining whether the nucleic acid sample contains sequence variations.

23. (Canceled) The method of claim 22 wherein said sequence variations are single nucleotide polymorphisms.

24. (Canceled) The method of claim 1 wherein the step of obtaining a DNA array comprises:

designing a DNA array to query DNA fragments which have been produced by the identical procedures used to obtain said second nucleic acid sample.

25. (Canceled) The method of claim 24 wherein the step of designing further requires predetermining the sequences contained in said second nucleic acid sample.

26. (Canceled) The method of claim wherein said step of predetermining the sequences contained in said second nucleic acid sample is conducted in a computer system.

27. (Canceled) The method of claim 23 wherein said second nucleic acid sample is obtainable by:

binding oligonucleotide probes containing a desired SNP sequence to magnetic beads to form probe-bead complexes; and

hybridizing said probe-bead complexes to said DNA sample;

exposing said hybridized DNA sample to a single strand DNA nuclease to remove single stranded DNA thereby forming a DNA duplex;

ligating a double stranded adaptor sequence comprising a restriction enzyme site to said DNA duplex;

digesting said DNA duplex with a restriction enzyme to release the magnetic bead; and

isolating only those fragments containing said SNP sequence.

28. (Canceled) The method of claim 25 wherein said restriction enzyme is a Class IIs endonuclease.

29. (Canceled) The method of claim 23 wherein said second nucleic acid sample is obtainable by:

exposing the DNA sample to a mismatch bonding protein;

employing a 3' to 5' exonuclease to remove single stranded DNA; and

employing a nuclease to remove single stranded DNA.

30. (Canceled) A method of screening for DNA sequence variations in an individual comprising:

providing said first nucleic acid sample from said individual;

providing a second nucleic acid sample by reproducibly reducing the complexity of said first nucleic acid sample to produce a second nucleic acid sample which may comprise a plurality of non-identical sequences whereby said second nucleic acid sample is obtainable by:

fragmenting said first nucleic acid sample to produce fragments and ligating adaptor sequences to said fragments;

fragmenting said first nucleic acid sample to produce fragments, denaturing said fragments, allowing some of said fragments to reanneal to form double stranded DNA sequences and removing said double stranded DNA sequences.

amplification by arbitrarily primed PCR;

hybridizing said first nucleic acid sample to an oligonucleotide probe bound to a solid support;

hybridizing said first nucleic acid sequence to a mismatch binding protein;

providing a nucleic acid array;

hybridizing said second nucleic acid sample to said array; and

analyzing a hybridization pattern resulting from said hybridization.

31. (Canceled) The method of claim 30 wherein said sequence variation is a SNP.

32. (Canceled) The method of claim 31 wherein said SNP is associated with a disease.

33. (Canceled) The method of claim 31 wherein said SNP is associated with the efficacy of a drug.

34. (Canceled) A method of screening for DNA sequence variations in a population of individuals comprising:

providing said a first nucleic acid sample from each of said individuals;

providing a second nucleic acid sample by reproducibly reducing the complexity of said first nucleic acid sample to produce a second nucleic acid sample which may comprise a plurality of non-identical sequences whereby said second nucleic acid sample is obtainable by:

fragmenting said first nucleic acid sample to produce fragments and ligating adaptor sequences to said fragments;

fragmenting said first nucleic acid sample to produce fragments, denaturing said fragments, allowing some of said fragments to reanneal to form double stranded DNA sequences and removing said double stranded DNA sequences.

amplification by arbitrarily primed PCR;

hybridizing said first nucleic acid sample to an oligonucleotide probe bound to a solid support;

hybridizing said first nucleic acid sequence to a mismatch binding protein;

providing a nucleic acid array;

hybridizing said second nucleic acid sample to said array; and

analyzing a hybridization pattern resulting from said hybridization.

35. (Canceled) The method of claim 34 further comprising the step of compiling the analyses of each individual's hybridization pattern.

36. (Canceled) The method of claim 34 wherein said sequence variation is a SNP.

37. (Canceled) In a computer system, a method of designing an array comprising:
modeling specific enzymatic reactions between a known nucleic acid sequence and an enzyme;
obtaining the results of said modeled enzymatic reactions;
obtaining probe sequences based upon said results; and
designing an array to contain said probe sequences.